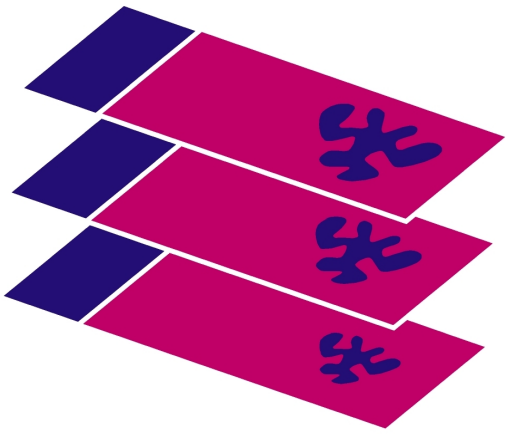

3DHISTECH

ImmunoScreener Module

1.12

User's Guide

March 03, 2009

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Font Types and Symbols

Example	Abbreviation or term that is explained in the section Terms and Abbreviations.
Example	Words or characters that appear on the screen. These include field names, screen titles, pushbuttons and menu names, paths or options. Keys on the keyboard. For example, function keys (such as **F11**) or the **Ctrl+O** key combination.
Example	Cross-references to other documents.
Warning!	Indicates that you need an additional module to use the feature or that there are prerequisites for the task or that special care should be taken when using certain feature of the device.
Important!	An important piece of information or a recommendation. The application will work if you choose not to follow the recommendation but its performance might be less than optimal.
Tip!	A suggestion about using the application in some other way or to some interesting purpose.

Terms and Abbreviations

Annotation	Area of interest on the digitized slide.
Consolidator	An additional user in a multi-user ImmunoScreener project who can see the scores given by all the other users and who can consolidate them.
Evaluator	A user who checks each field in each grid on each slide and assigns it a score. In a multi-user ImmunoScreener project, evaluators do not see each other's scores.
Field	A cell of the grid that is fitted onto an area of interest on the virtual slide. If you put a grid on slides from the same specimen, you can analyze the marker expression of the same fragment immunostained for different antigens. ImmunoScreener projects allow you to correlate the different antigens and perform measurements (for example with a separately available MIRAX Viewer module, HistoQuant).
Grid	A set of annotations used in ImmunoScreener. They are independent of other annotations, for example HistoQuant annotations that might be smaller than a grid field. Unlike plain MIRAX annotations or HistoQuant annotations, grids are not saved with the slides by default. Grid information is stored with the ImmunoScreener project where you created or modified them but you can also save them on the slides.
ImmunoScreener Gallery	An ImmunoScreener gallery contains all fields from all grids from all slides in the project. For example, if you used a 2x3 grid and marked an area with the grid on 3 slides, the gallery for this stain on this specimen contains 2x3x3=18 images. In gallery mode, you can magnify small parts (the grid fields) to score them and to export them for reporting or in an image gallery.
ImmunoScreener project	An ImmunoScreener project is a set of slides that you want to evaluate with each other. In multi-user ImmunoScreener projects only the Consolidator can modify the project (add or remove slides, add or remove stains).
IHC	Immunohistochemistry.
Marker	A stain, for example hormone (Estrogen-Receptor, Progesteron-Receptor, Her-2, EGFR, etc.).
Stain	Classic pathological stains, for example HE.

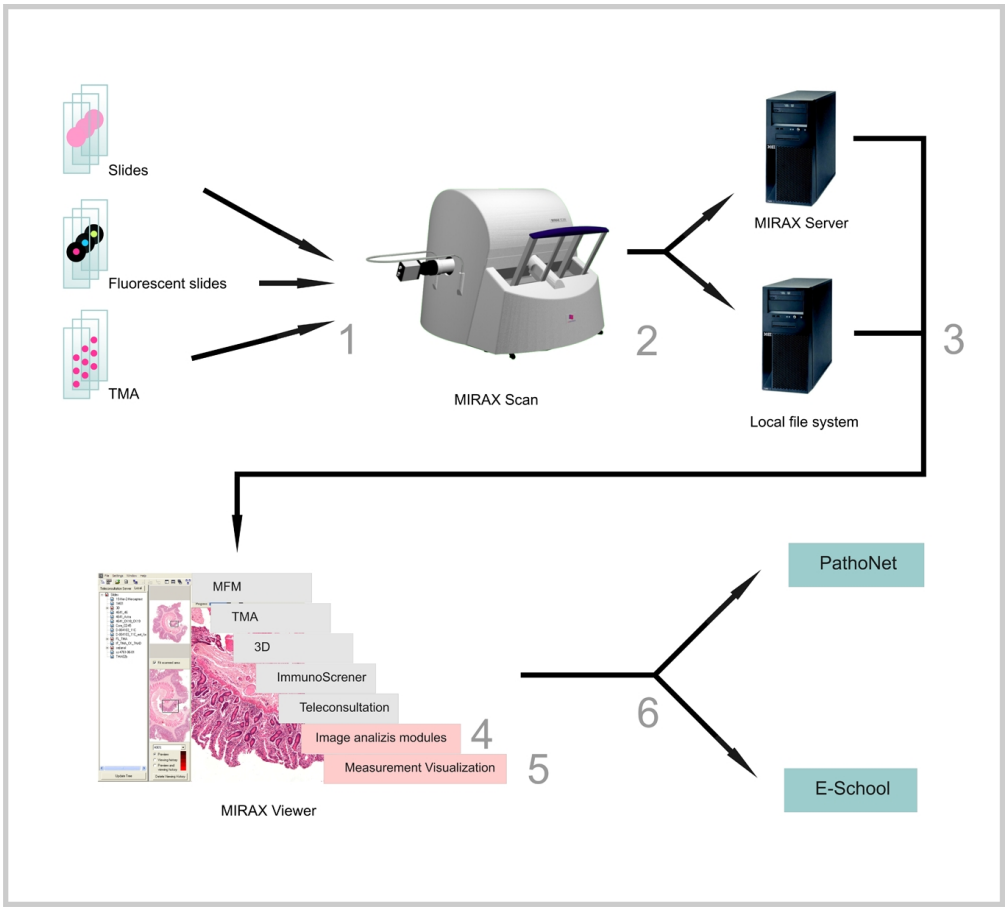
1 About the ImmunoScreener Module 1.12

ImmunoScreener is an optional module for MIRAX Viewer. It allows you to perform (semi)quantitative histopathological examinations of *IHC-stain*ed slides, digitized with MIRAX scanners.

- In the routine pathology, you can use **ImmunoScreener** to check specimens dyed with common *IHC stains*. Such an **ImmunoScreener** *project* typically contains sequential cuts from the same specimen and each slide is treated with a different *stain*.
- Research projects often use **ImmunoScreener** to check cuts from different specimens, for example from biopsies from the same patient, taken at different points in time. In such cases, the slides would be dyed with the same *marker*.

1.1 ImmunoScreener Module in the MIRAX Product Family

For an overview of the workflow, see ***MIRAX Viewer - User's Guide***.



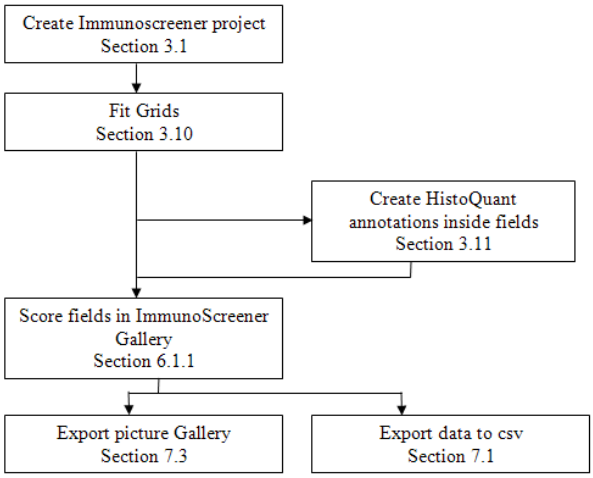
With the **ImmunoScreener** module, you can perform an immunohistochemical analysis on a slide set digitized with MIRAX scanners and stored on a local file system or on a MIRAX Slide Server.

1.2 Overview of the ImmunoScreener Module 1.12

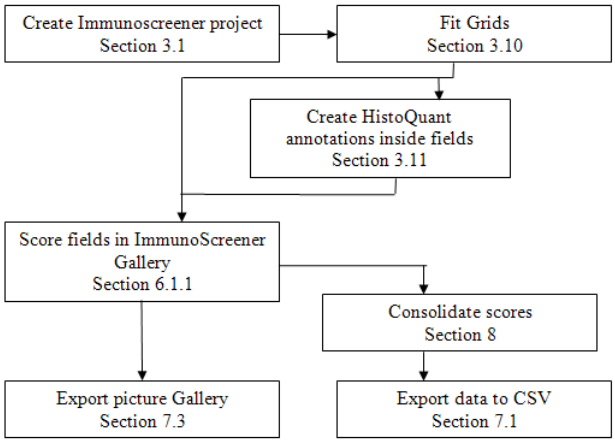
ImmunoScreener supports decision-making in targeted therapy or in *marker* validation. It also makes your evaluations more reliable. With **ImmunoScreener**, you make subjective slide scoring more accurate and you make it measurable, because you compare areas and scores on digitized MIRAX slides, which is a more reliable method than analyzing the actual slides empirically under traditional microscopes.

1.2.1 Workflow

The following figure summarizes your workflow:



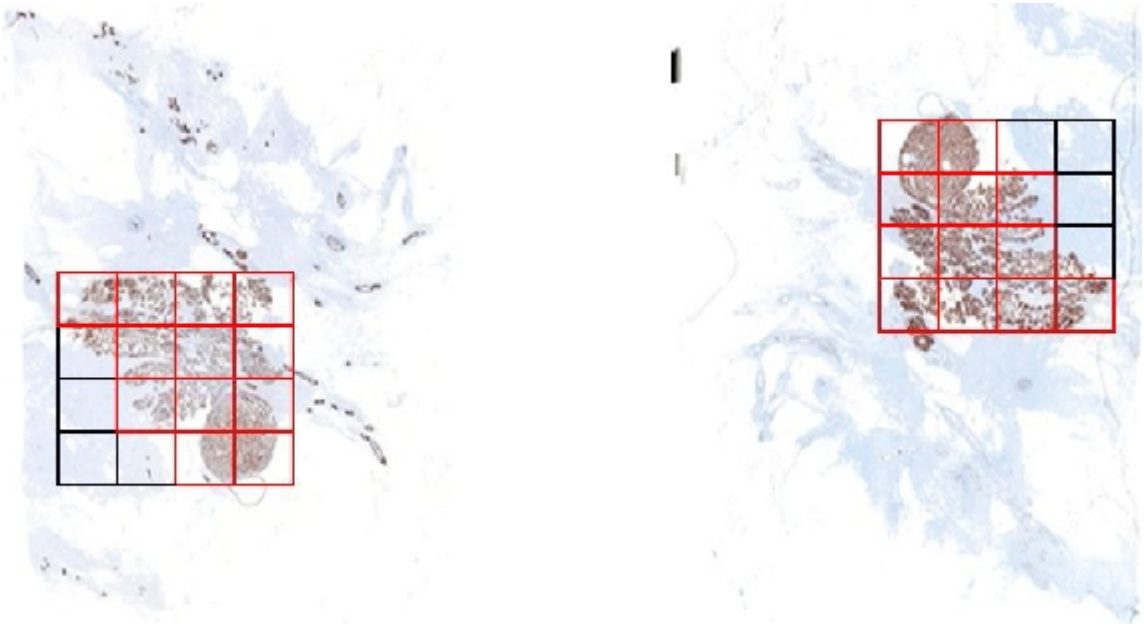
You can also use **ImmunoScreener** together with your colleagues. In this case, each team member scores the slides, and then one team member consolidates the scores.



For instructions on how to consolidate scores, see section 8.

1.2.2 Grids

In routine pathology, an **ImmunoScreener** *project* contains slides from the same specimen. In such cases, *grid*s are typically put on the same area on each slide, for example on a group of cancer cells.



Typically used *grid* setups:

n x m grid	You can put a *grid* on the area of interest and obtain data about smaller areas (*grid field*s). You can fit *grid*s on the same area on several slides. **ImmunoScreener** helps you rotate and align the *grid* throughout several cuts from the same specimen. This allows you to obtain a report at object level (for example, about the nuclei found in the *field*) and also at the level of the *field* (for example, the number of positive cells compared to the number of negative cells). You can also knock out *field*s which are not interesting or not needed for the evaluation (for example, no tumor cells in it)
1 x 1 grid	For better sampling from the specimen if one n x m *grid* is too large
Several grids	You can put several *grid*s on the slides (scoring is done per *annotation*, that is, per *grid field*, **ImmunoScreener** calculates the final score per slide)
Grid-internal annotations	You can also create *annotation*s that are smaller than the *grid field*s. You can use *grid annotation*s and non-**ImmunoScreener** *annotation*s on the same slide without any problem. If you want to use **HistoQuant**, you can run **HistoQuant** algorithms on either type of the *annotation*s.
Grids on several slides in the project	You can analyze several slides in a project and get a better overview of a specimen. Furthermore, you can export the data into one report.

1.2.3 Scoring Schemes

You can define a separate scoring scheme for each *stain* and several users can evaluate the slides. A *Consolidator* user reviews the scores and **ImmunoScreener** computes the final score for each *annotation* (each *field* in each *grid* on each slide).

1.2.4 Reusing Stain Definitions and Scoring Schemes

You can reuse *stain*s and scoring schemes defined in other **ImmunoScreener** *project*s. The definitions are saved in a file (IHLabConfig.xml) and you can copy them to the machine of other MIRAX Viewer users.

1.2.5 Multi-User Mode and Score Consolidation

ImmunoScreener can handle scores from several *evaluator*s and average their results. It also creates a *Consolidator* user for each multi-user report to facilitate score consolidation.

1.3 ImmunoScreener and HistoQuant / Measurement Visualization

You can also use **ImmunoScreener** with other MIRAX Viewer modules:

Warning!

Each MIRAX Viewer module is licensed separately.

- With **HistoQuant** - the module that allows you to define and run measurement algorithms on MIRAX *annotation*s - you can perform the measurements on the entire slide set in an **ImmunoScreener** *project*.
- With **Measurement Visualization**, you can display the **HistoQuant** measurements as a scatterplot, a histogram, or as a gallery of pictures. You can also export your results into a CSV file or as an HTML picture gallery.

2 Installing the ImmunoScreener module for MIRAX Viewer

2.1 Licensing of MIRAX Products

Mirax software is purchased with a valid license file (MView.lic) which the software needs to exploit its all options. Without this file the Mirax Viewer offers only limited options as a free Mirax Viewer.

2.2 Prerequisites

ImmunoScreener is not a standalone application, you need MIRAX Viewer to run it. Therefore, it is installed together with MIRAX Viewer, bundled into one executable.

MIRAX Viewer version	1.12 Running on Microsoft Windows XP
Hardware	**minimum**: Pentium P4, 3GHz, 1GB RAM **recommended**: Pentium C2D (DualCore), 2 GHz, 2GB RAM
Disk space	MIRAX Viewer bundled with the **ImmunoScreener** module **minimum**: 88 MB

2.3 Installing the Application Bundle

1. Start the MIRAX Viewer **setup.exe** file that you received on a MIRAX Viewer DVD or downloaded in a compressed file.
2. Follow the instructions in the installation wizard.
3. Start the application **setup.exe** file that you received on the application DVD or downloaded in a compressed file.
4. Follow the instructions in the installation wizard.
5. For more information, see ***MIRAX Viewer 1.12 Installation Guide***.

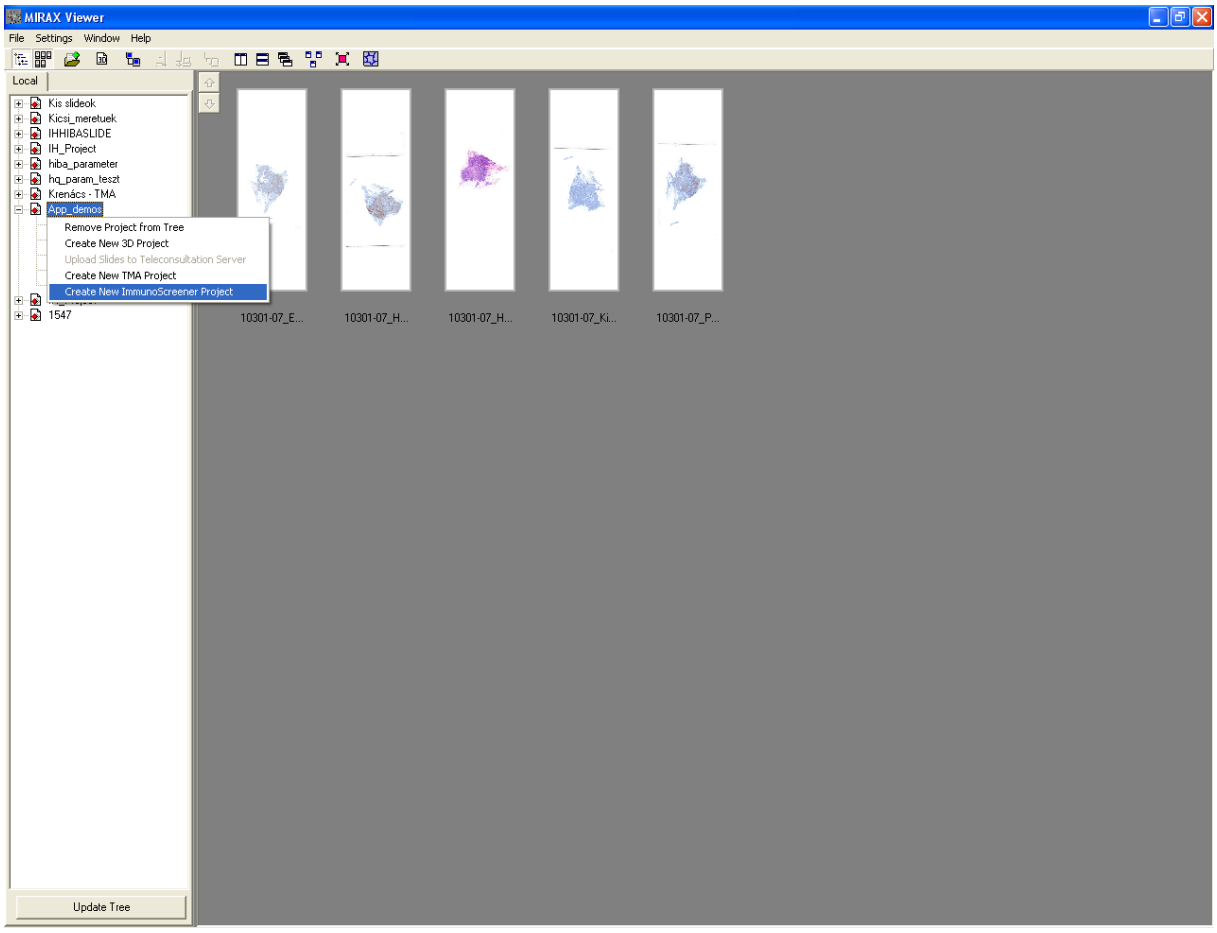
3 Creating an ImmunoScreener Project

Before You Start

Make sure that the slides that you want to add to the **ImmunoScreener** *project* are all in one folder at a location that you can access from MIRAX Viewer.

3.1 Create New ImmunoScreener Project

ImmunoScreener *project*s are handled and created within Mirax Viewer. In the **Project Tree**, right-click the folder where you want to create the **ImmunoScreener** *project*, select **Create New ImmunoScreener Project** from the menu and enter a name for the new project.



ImmunoScreener *Project* Wizard is launched and helps you go through all the necessary steps.

3.2 Name the New ImmunoScreener Project

As it cannot be modified later, be sure to incorporate all the necessary information into the name of the new project.

The **ImmunoScreener** *Project* will be located within the folder of its slides, which can be visualized after pressing **Update Tree** in the Mirax Viewer screen.

3.3 Define at Least One User on User Management Screen

Enter a user (name and ID) who will score the slides.

If you need a multi-user project, you can add further users later on, when the project has been created and you first start the project.

3.4 Project Data

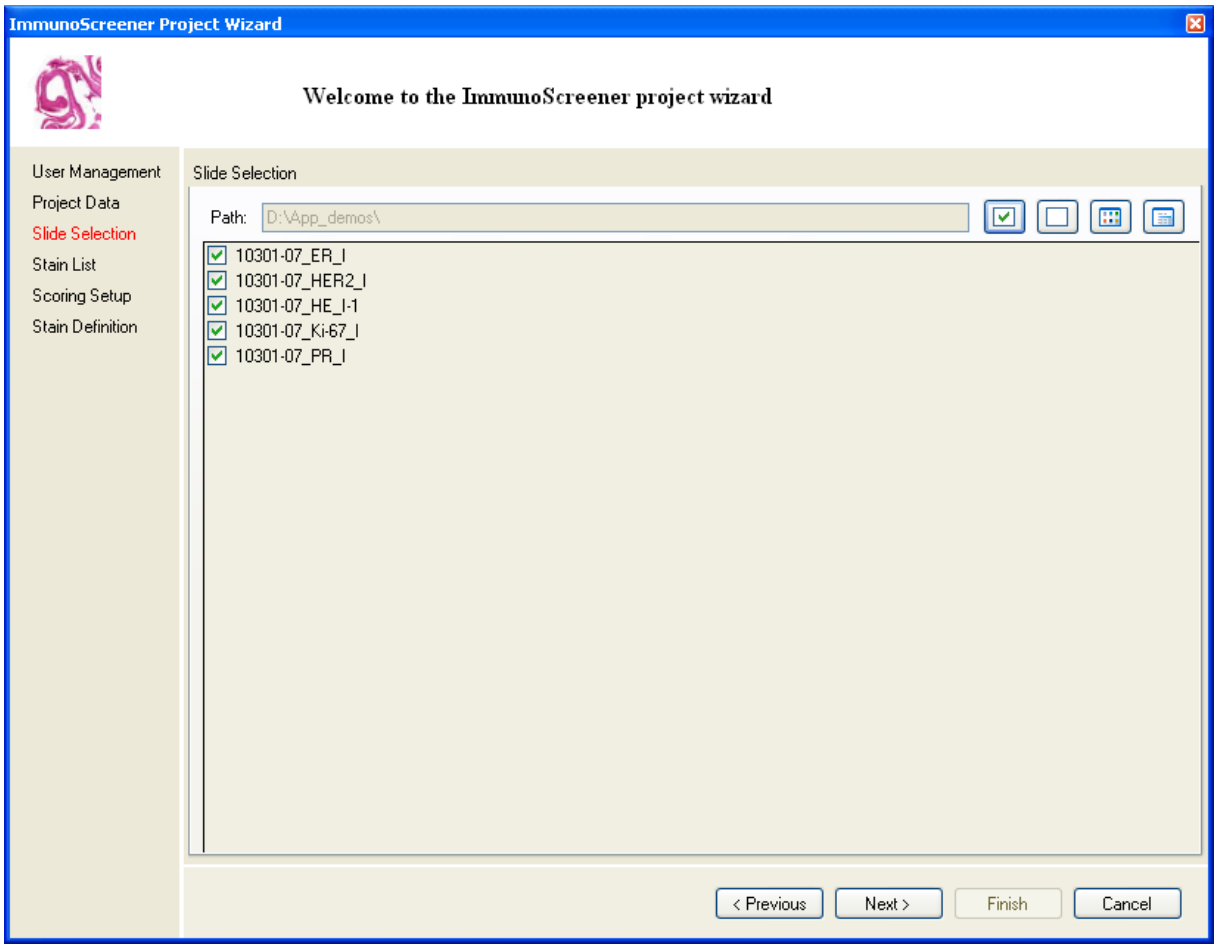
You may add parameters and values to the project, for example patient name(s) and the corresponding IDs or registry numbers, if it is needed. You may skip this option.

3.5 Slide Selection

You can see all slides in the actual folder. Select or clear checkboxes to create the slide set that you need for this **ImmunoScreener** *project*.

For multiple selection you can select several slides at the same time with **Shift+Click** and/or **Ctrl+Click**. (This option is also valid for the further steps in the Wizard.)

The slides that you leave out are not removed from the file system. You can also add them or new ones to the project later.



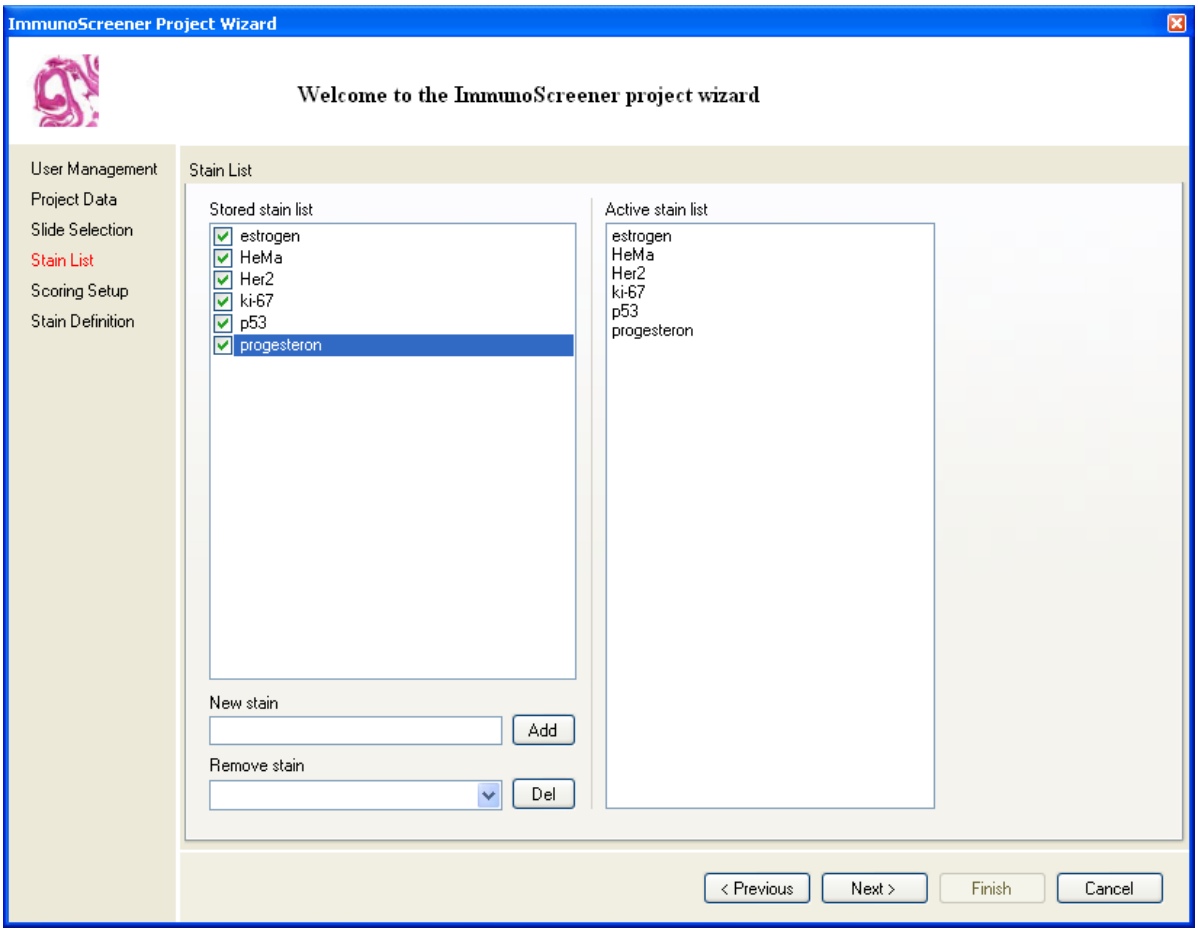
allows you to view the thumbnails of the slides.

allows you to view the list of the slides.

3.6 Stain List

Specify which *marker*s are used in the actual project. Use checkboxes to add *marker*s to the *marker* list.

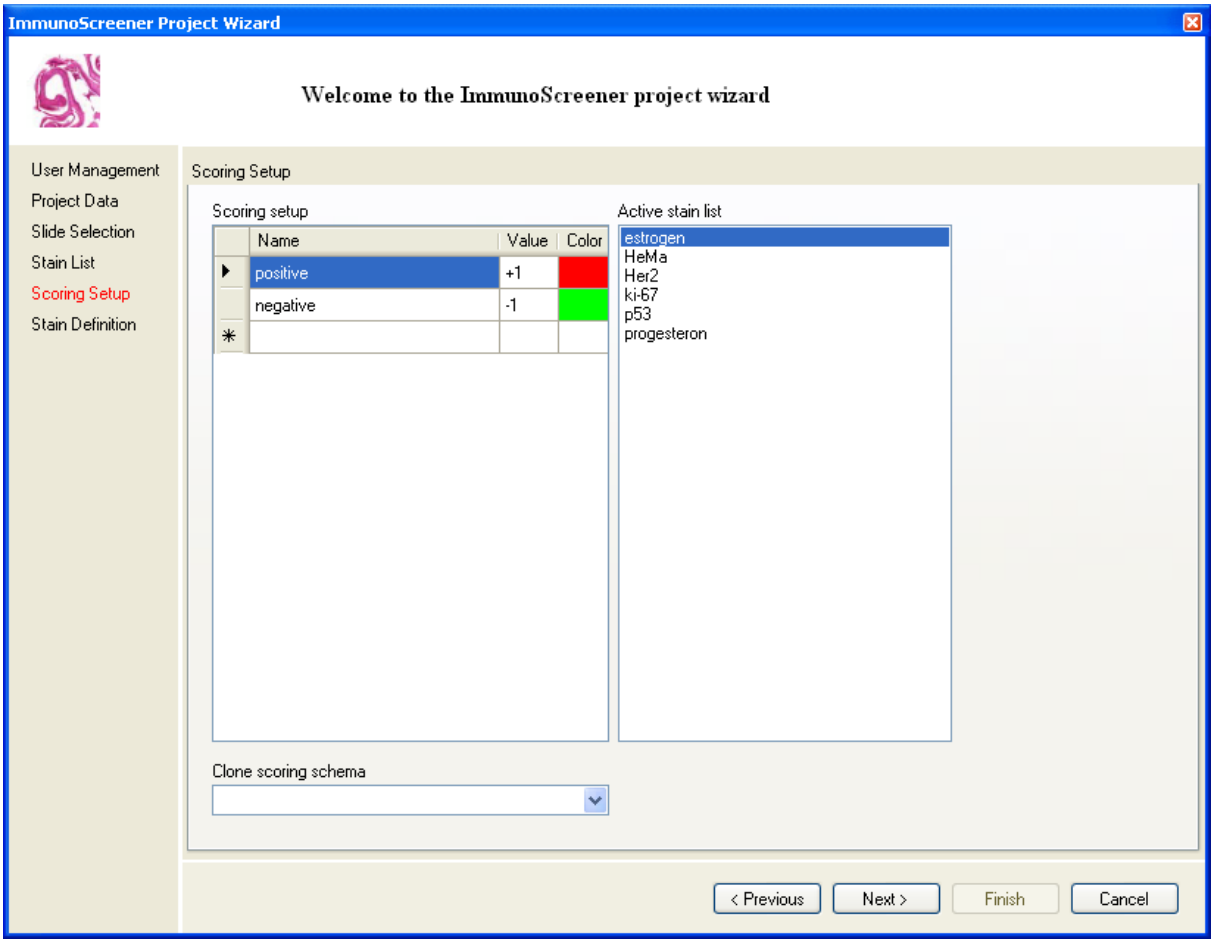
You can also add new *stain*s here.



3.7 Scoring Setup

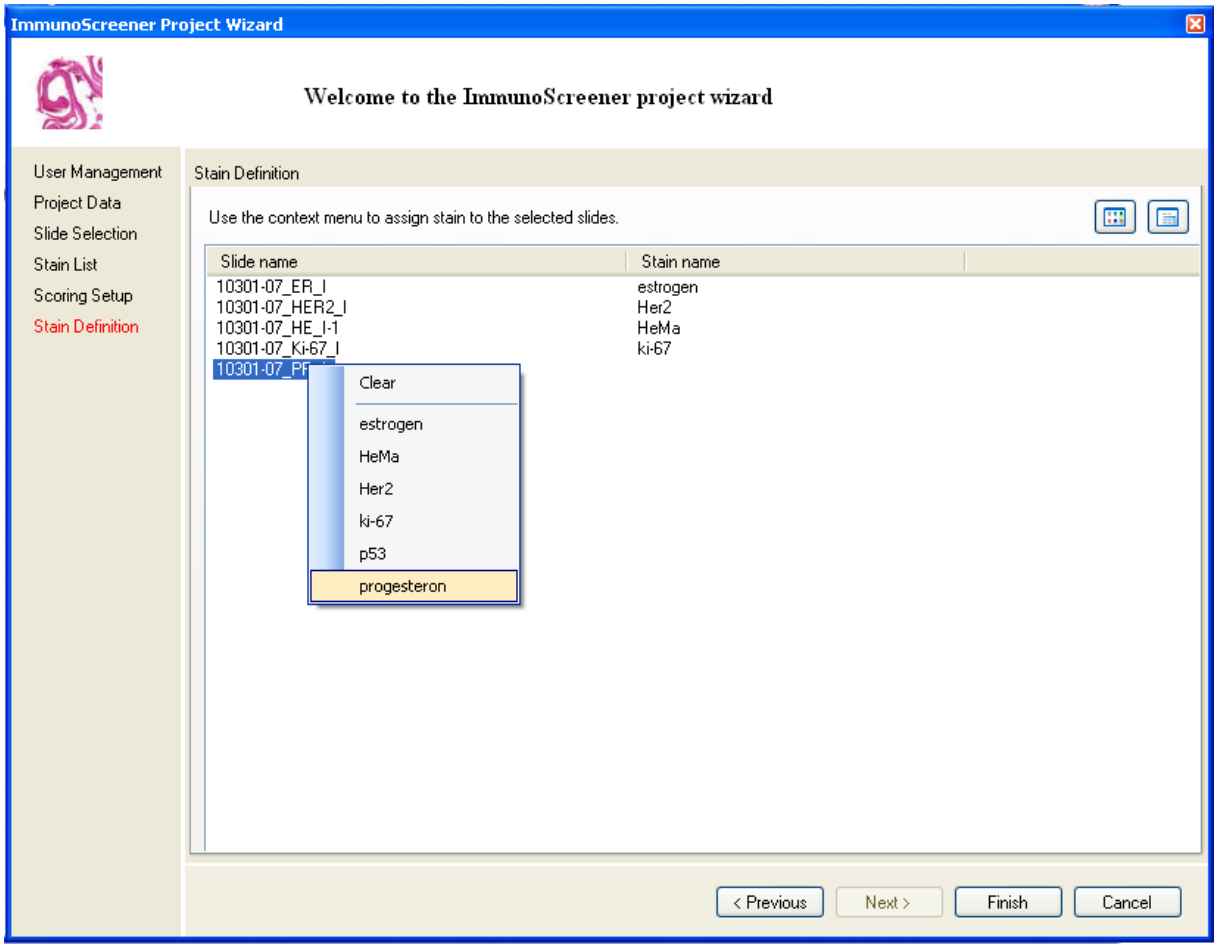
Create a scoring scheme for the *stain*s. You can assign a different scheme (numeric value and color) to each *stain*. You can also speed up scheme generation by cloning or reusing existing schemes.

You can also use the available scoring schemes. If you modify them for a *stain*, the scoring scheme is saved with the changes for this *stain*, but it is not changed for the "old" *stain*s.



3.8 Stain Definition

Mark for each slide which *stain* is applied on it. You can select several slides with **Shift+Click** and **Ctrl+Click** and apply the same *stain marker* on them in one step.



The wizard asks you whether you want to start fitting the *grid* onto the slides right away. You can do *grid* fitting later. To start *grid* fitting right away, select the checkbox in the pop-up window.

3.9 Grid Definition

Important!

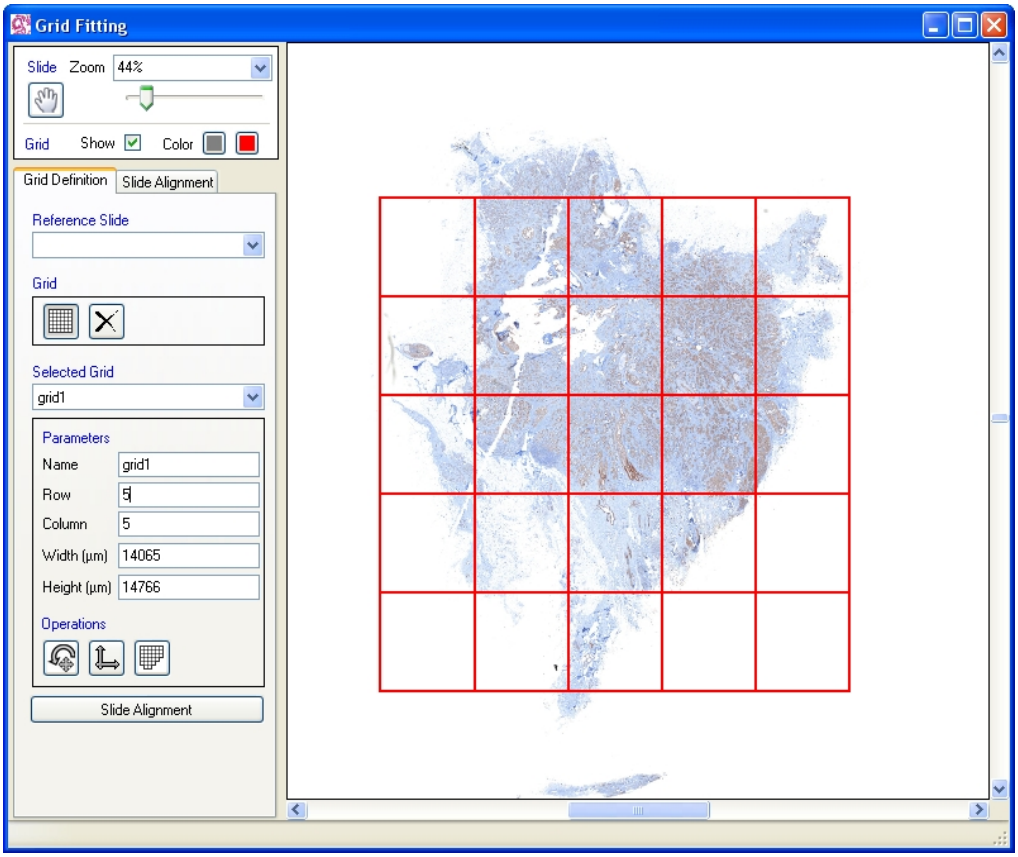
In multi-user projects, only the *Consolidator* user can fit *grid*s to the slides.

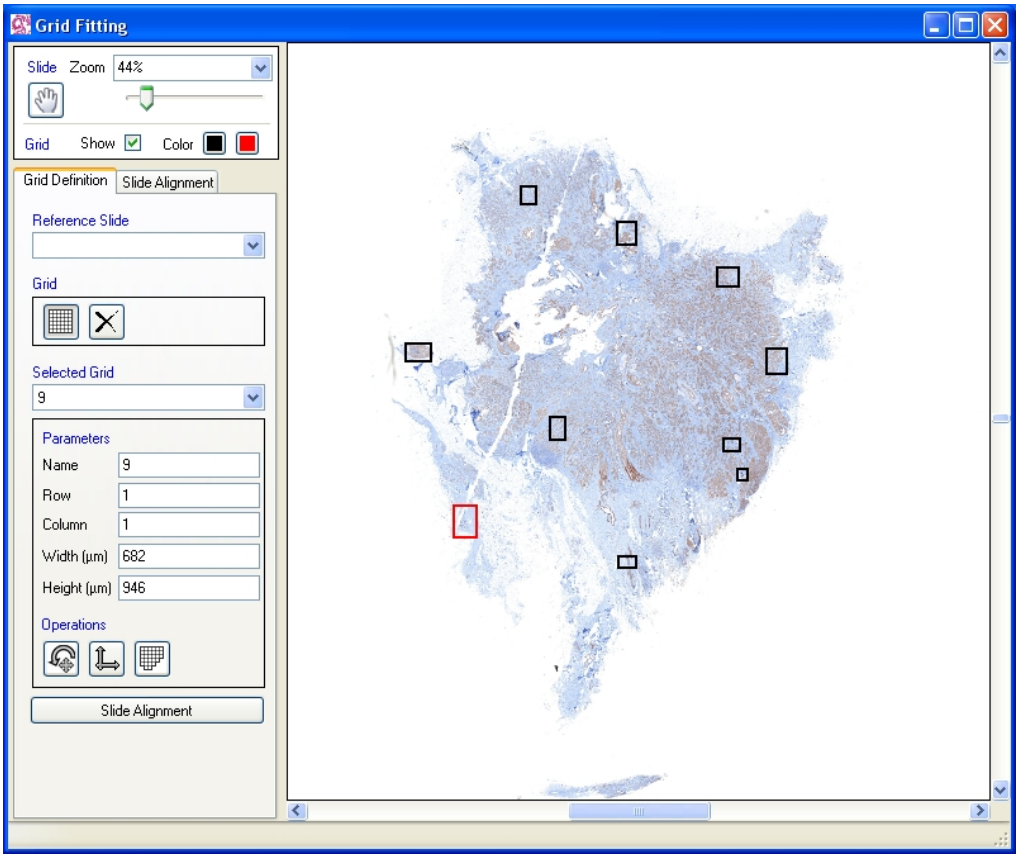
On each slide, mark the areas that you want to inspect with one or more *grid*s, for example areas with suspected cancer.

Specify the *grid* properties: name, number of rows and columns, the size of the *grid* in µm.

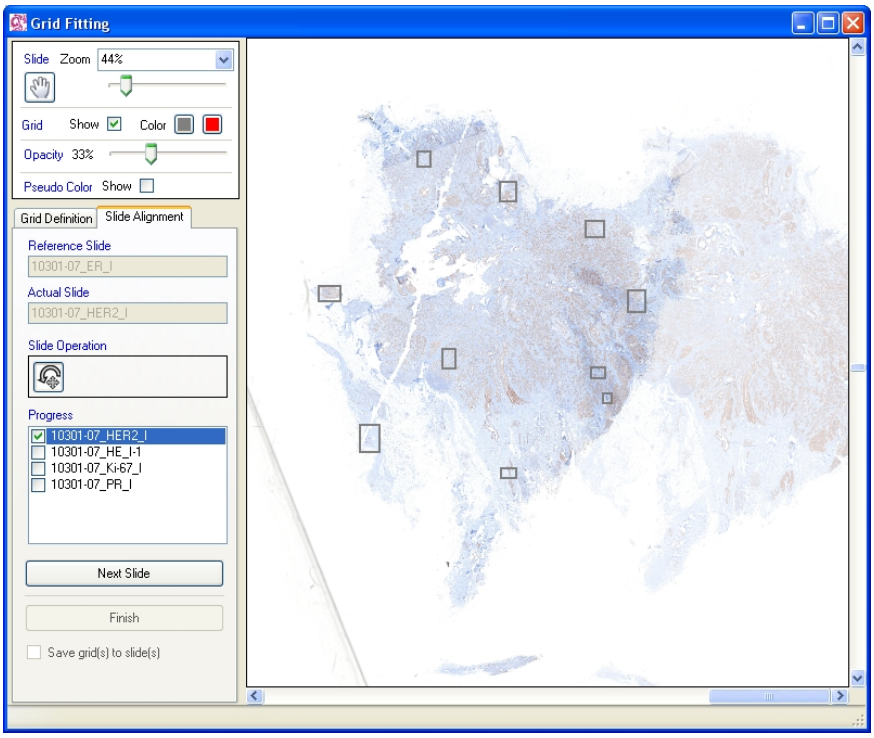
You can also adjust the position of the *grid* on the slide, or rotate, enlarge / shrink it by dragging the *grid* borders.

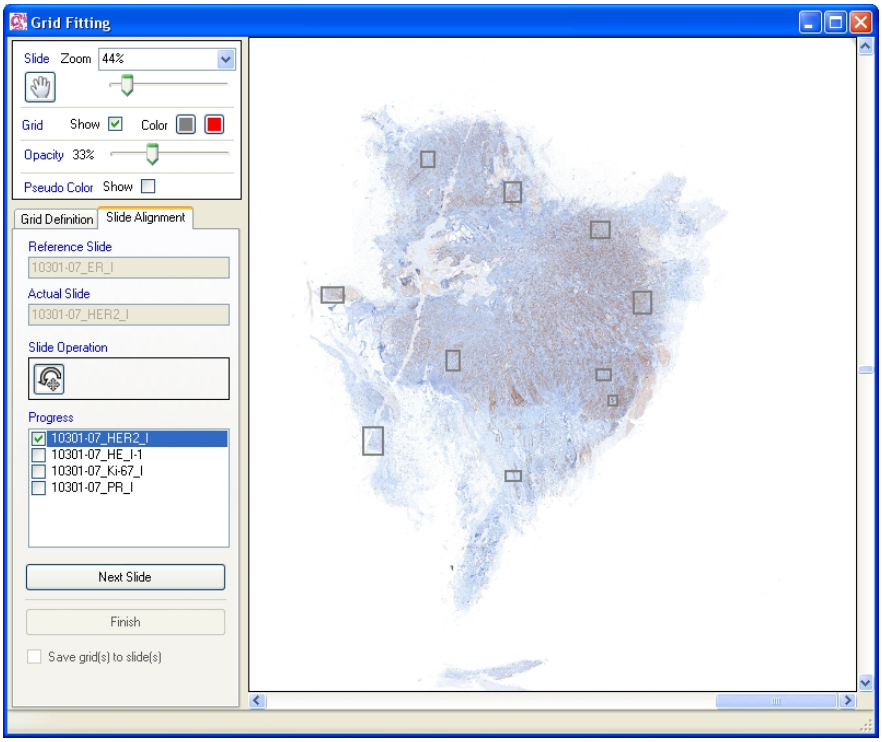
You can also put several *grid*s on the slides by clicking **New Grid**.




Grid Fitting
Slide
Zoom
44%
Grid
Show
Color
Grid Definition
Slide Alignment
Reference Slide
Grid
Selected Grid
9
Parameters
Name
9
Row
1
Column
1
Width (µm)
682
Height (µm)
946
Operations
Slide Alignment

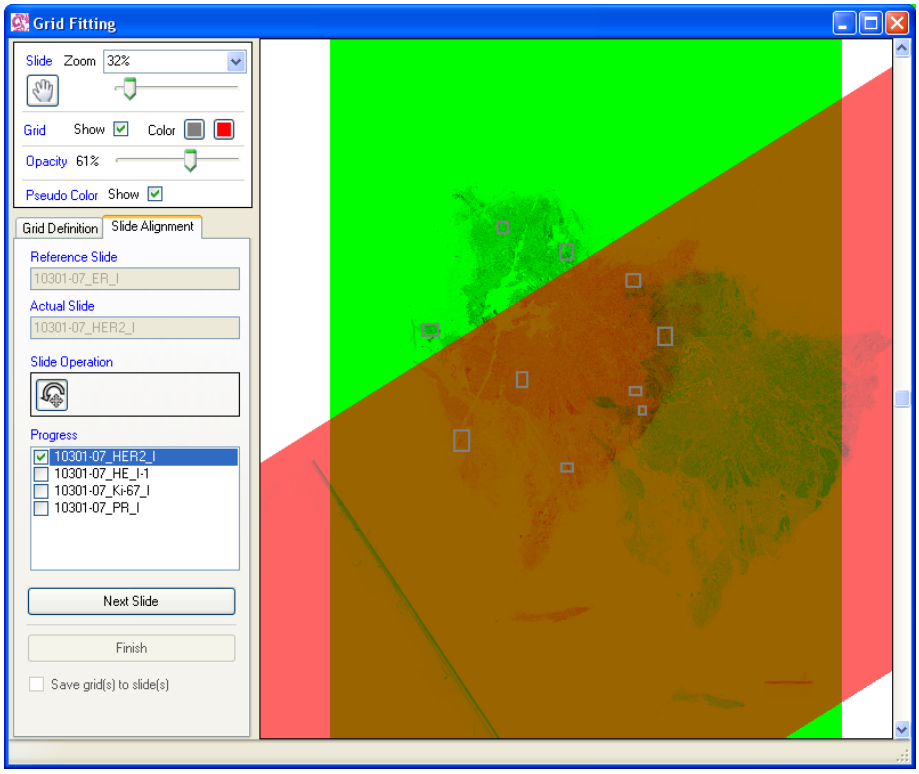
3.10 Grid fitting

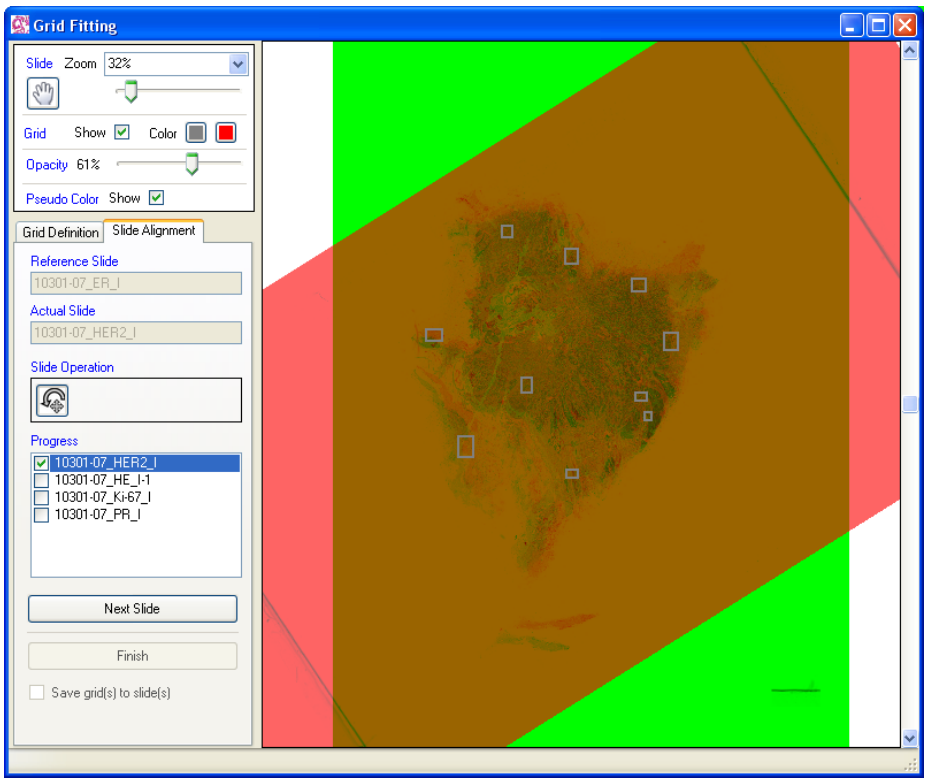




Go through all slides pairwise and align them onto each other. Move (left mouse button) or rotate (right mouse button) the slides so that the *grid*s cover exactly the same area on the other.

Using **Pseudo Color** option helps you to better adjust slides.





Repeat these steps for each slide that you want to use in this **ImmunoScreener** *project*.

Save the *grid* to the slide if you want to further analyze *annotation*s with **HistoQuant**.

Click **Finish** to create the **ImmunoScreener** *project*.

3.11 Creating Annotations Inside an ImmunoScreener Grid

ImmunoScreener *grid*s are *annotation*s that you create when you create the **ImmunoScreener** *project*. You can nevertheless use further *annotation*s on the slides. There might already be *annotation*s on a slide or you might want to create **HistoQuant** *annotation*s that are smaller than the *grid field*. If you have the optional **HistoQuant** module, you can perform measurements on the **HistoQuant** *annotation*s.

1. Open the slide in MIRAX Viewer.
2. Create the *annotation* on the area of your interest:
    a. Select the *annotation* type (rectangle, free-hand or point) and cover the area with the *annotation*.
    b. Add a caption to the *annotation* and enter a description.

       If you are using the **HistoQuant** module, note that only captions are exported to the CSV output file. *Annotation* descriptions are for internal use in MIRAX Viewer and **HistoQuant**.
3. Repeat step 2 for each area you want to run the algorithm on.

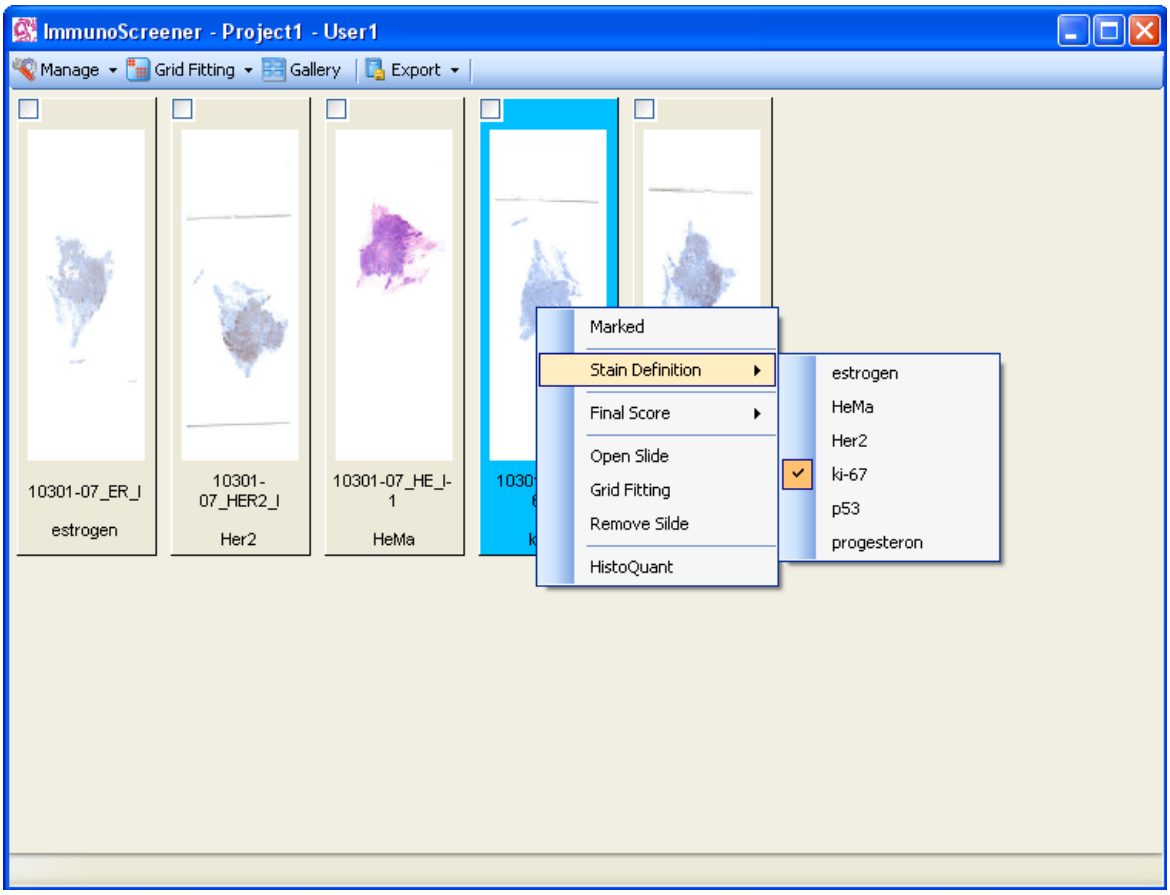
   You will be able to run other algorithms on these *annotation*s.

4 Handling an ImmunoScreener Project

4.1 Options in the ImmunoScreener Project

You can manage slides and *stains*; perform *grid* fitting, open gallery and export options from **ImmunoScreener Toolbar,** or by directly choosing from the options by right-clicking on the slide.

You can also mark slides, give a final score or open the slide or the **HistoQuant** module.



4.2 Modifying an ImmunoScreener Project

Important!

If you modify an **ImmunoScreener** *project*, the scores related to the slide/*stain* or *grid* are lost for all users who have already submitted scores to the project.

Most of the settings of an **ImmunoScreener** *project* can be modified.

In 'Single-user' mode, the user can modify the project, but in 'Multi-user' mode, only the C*onsolidator* user can modify **ImmunoScreener** *project*s:

- slides that are included in the project (add or delete slides)
- *stain*s that are used in the project (add or delete *stain*s)
- number of *grid*s that are used in the project (add or delete *grid*s)
- *grid* layout (modify the size of a *grid*, change the number of rows or columns, knock out *field*s or put *field*s back into the *grid*).

4.3 Adding or Removing Slides

The way of adding or removing slides from the *ImmunoScreener project*:

1. Start the **ImmunoScreener** *project*.
2. Select **Manage > Add / Remove Slide(s)**.
3. Add or remove slides as needed:
    a. Browse for slides to add.
    b. Clear the checkbox of the slide(s) that you want to remove.
4. Click **OK** to update the **ImmunoScreener** *project*.

The way of removing a slide quickly:

1. Start the **ImmunoScreener** *project*.
2. Right-click the slide to be removed and select **Remove Slide**.

4.4 Adding or Removing Stain*s*

1. Start the **ImmunoScreener** *project*.
2. Select **Manage > Stain Manager**.
3. Add or remove *stain*s as needed and click **OK**.

4.5 Changing the Grid

Important!

In multi-user projects, only the *Consolidator* user can fit or modify *grid*s.

1. Start the **ImmunoScreener** *project*.
2. Start the **Grid Fitting** wizard:
   a. Select **Grid Fitting** and select the appropriate option, either **For All Slides** or **For Selected Slides**.
   b. Right-click a slide that you want to change the *grid* of, and select **Grid Fitting** from the shortcut menu.
3. Modify the *grid* as needed.
4. Save the changes by clicking **Finish** on the **Slide Alignment** tab.

5 Working with ImmunoScreener Gallery

5.1 Enlarging the Preview Window

To make the preview window as large as possible, you can hide the panel in the **ImmunoScreener** gallery you are working on by clicking the border between the panel and the preview window.

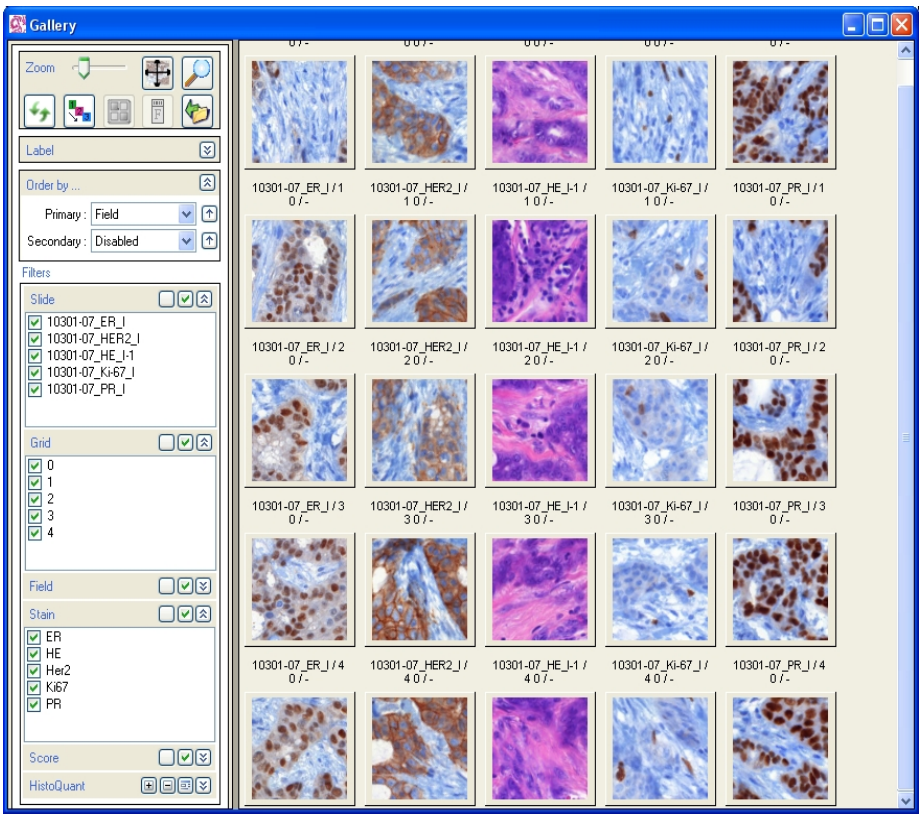
5.2 Display Options of ImmunoScreener Gallery

In the gallery, you can determine the size of the preview of the *field*s by setting **Zoom**. The gallery provides multiple visualization options for the ImmunoScreener *project*, which you can change at the left side of the window. By default, ImmunoScreener shows the *annotation*s (the *grid field*s) as thumbnails in the following order of importance: group by slides, group by *grid*s, group by *field*s.

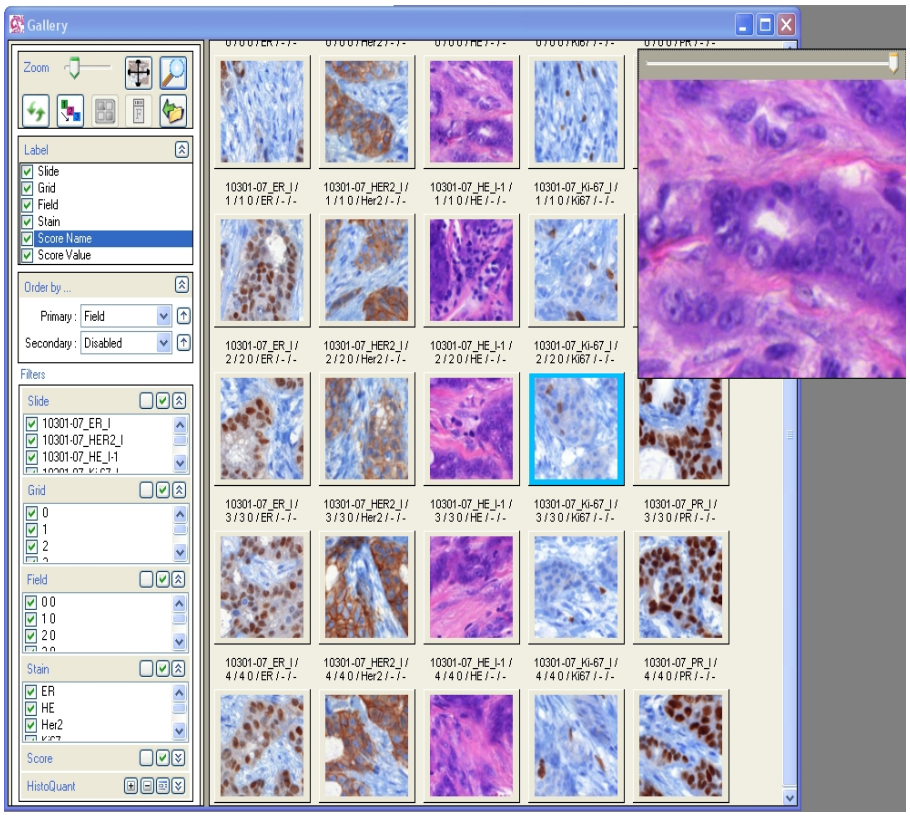
To modify the display order, set the primary and secondary ordering principle in the **Order by...** area and click to refresh the gallery.

If you are evaluating an **ImmunoScreener** *project* from the same specimen, where the slides were handled with different *stain*s, order the thumbnails by **Stain**.

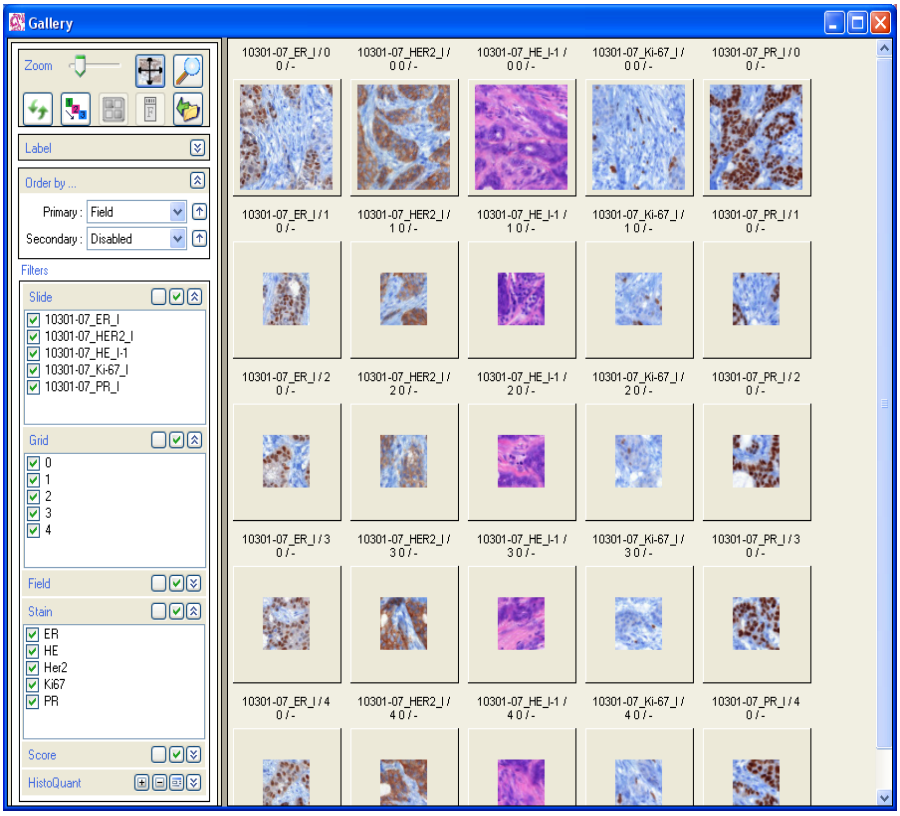
If you want to check your consistency in scoring, order the thumbnails by **Score**.



You can zoom in / out on the selected field previews by left-clicking on the window, holding down the button and moving the glide.

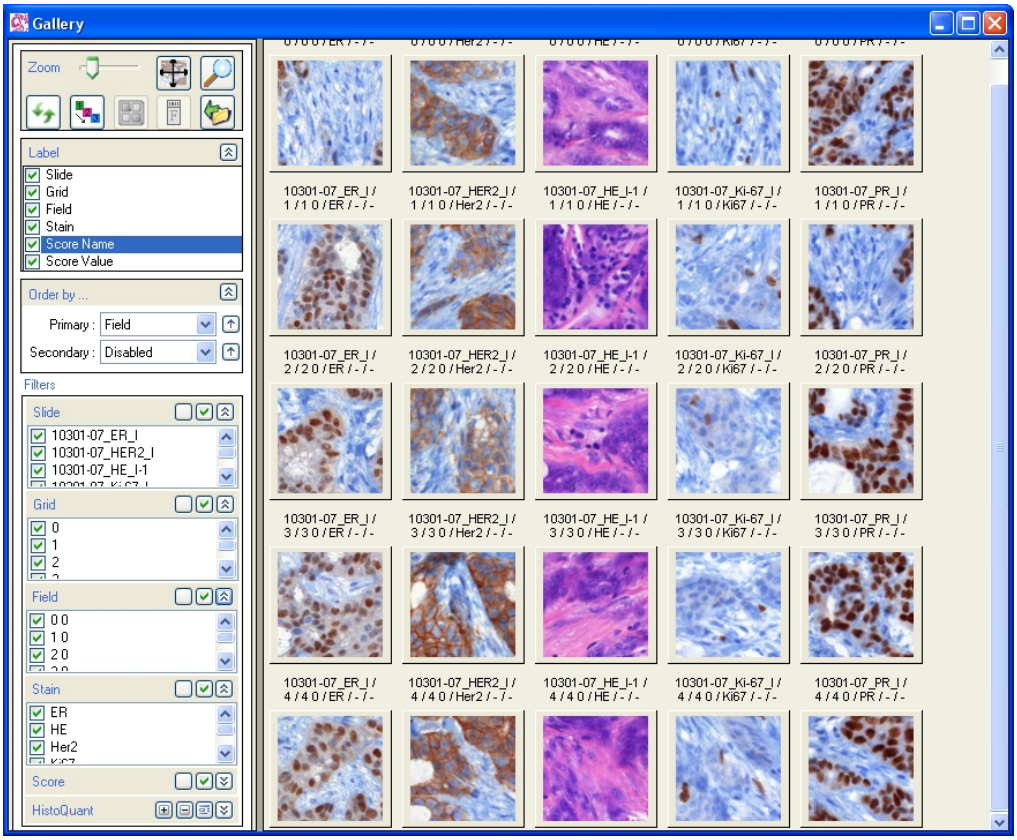


You can also enlarge the preview objects within their frames by using **Cell Fitting**, but the size of the gallery and the zoom will not change.



5.3 Setting Which Labels Are Visible in the HTML Export

You can change the content of the captions visible above the *field*s in the **Immuno-Screener** *Gallery*. For example, you might want to show the slide number and the *grid field* number in the caption of the thumbnails if you plan to export an HTML gallery from your **ImmunoScreener** *project*, because this is the only way to show the scores in the output.



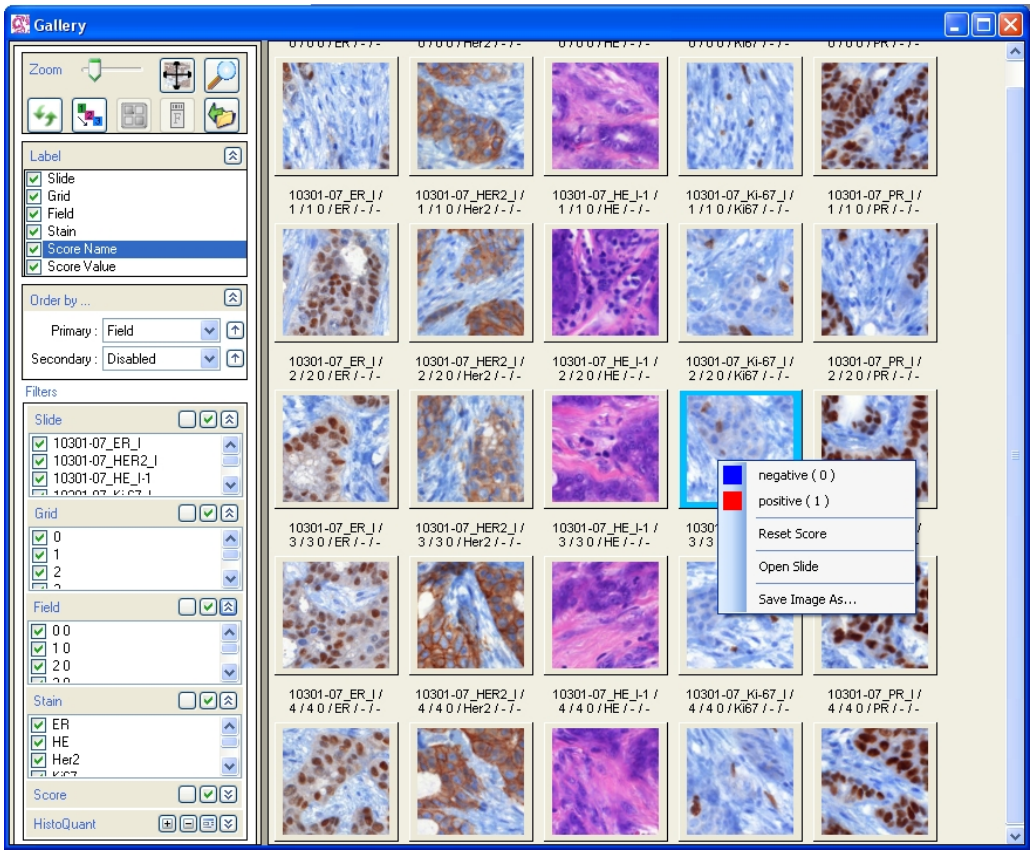
6 Scoring Slides in an ImmunoScreener Project

6.1 Scoring Options:

- From the gallery
- Sequential scoring
- Per slide

6.1.1 Scoring in the ImmunoScreener Gallery

1. Open the **ImmunoScreener** *project*.
2. Click **Gallery** in the project menu and display the thumbnails.
3. Score the *grid fields*:

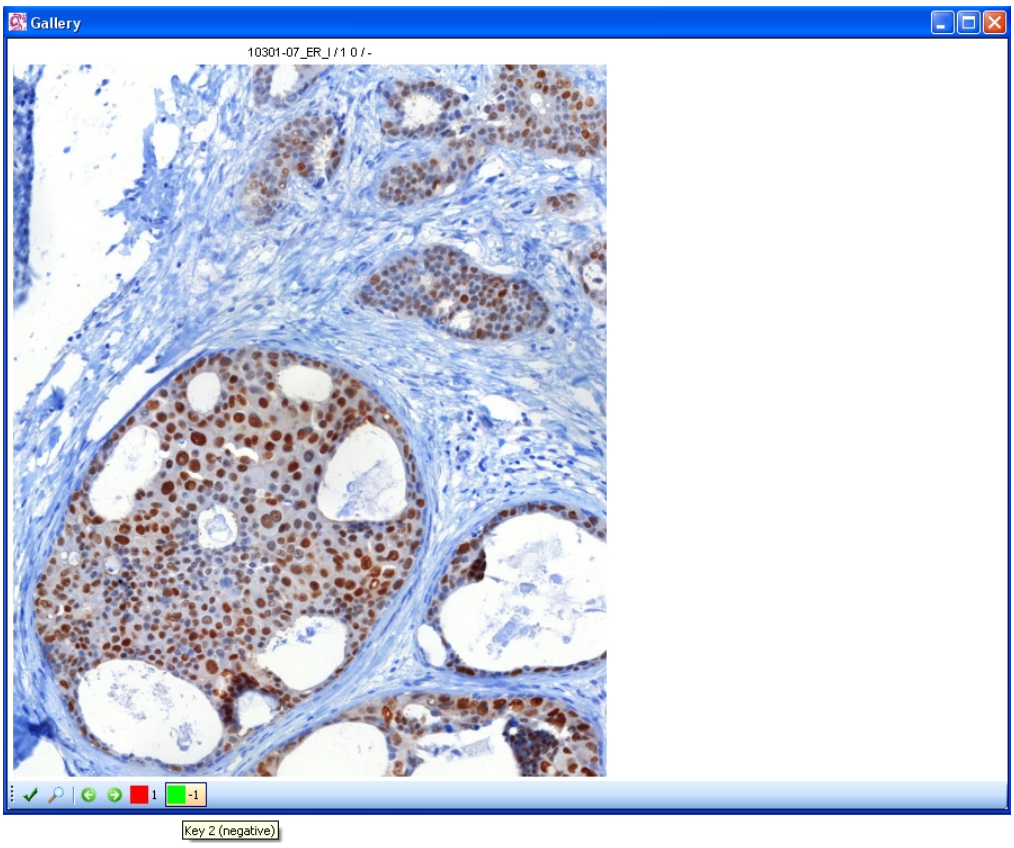


- To change the order of the display before you start scoring, change the grouping settings in the **Order by** frame.

- To score the *annotation*s in the order they are displayed in the gallery, start sequential scoring.
- To score the *annotation*s one-by-one, right-click each thumbnail and select the score from the shortcut menu.

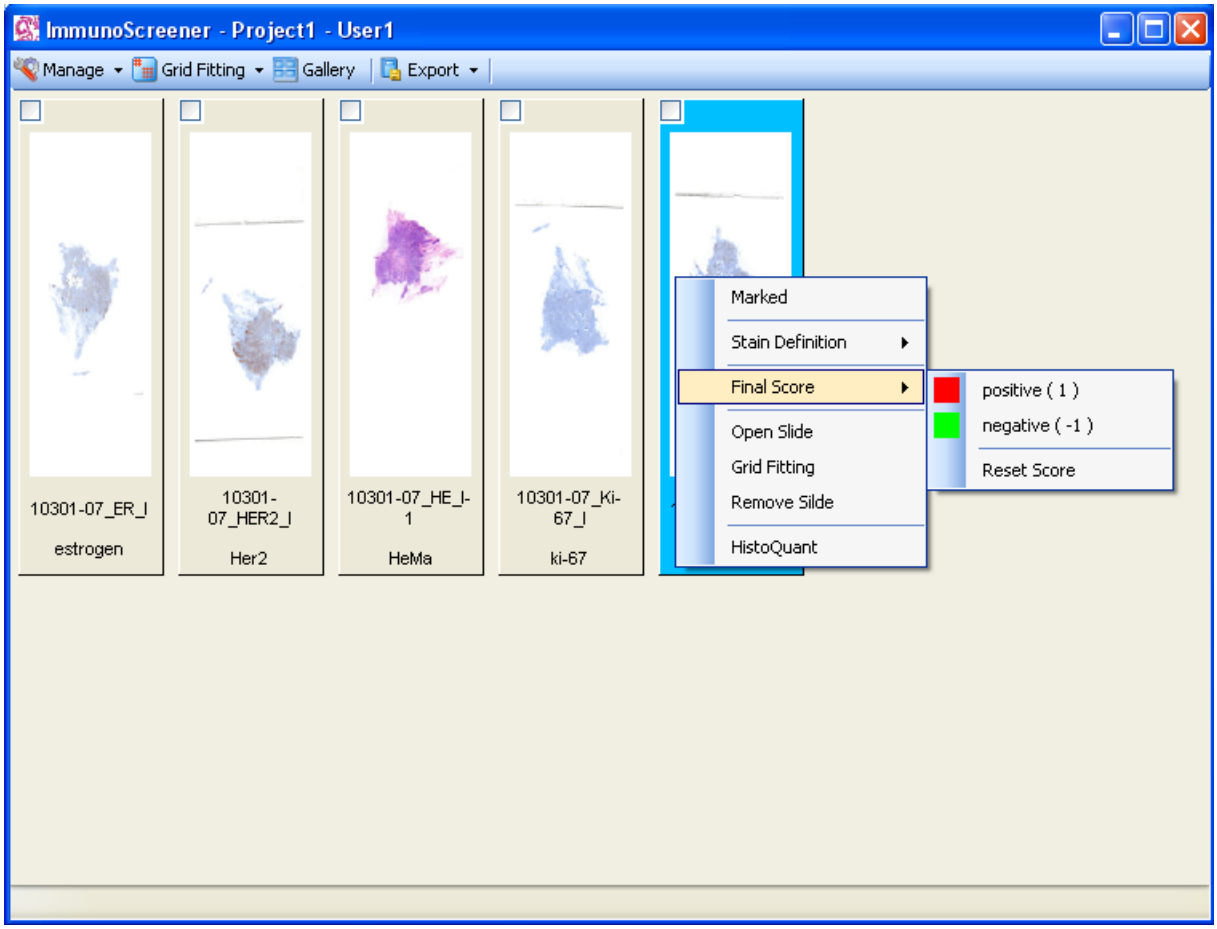
6.1.2 Sequential Scoring

Sequential scoring is a comfortable way to go through and score each *field* of the *grid*s. You can launch this option by clicking in the Gallery. Only one *field* appears in the window for evaluation, and by clicking the score at the bottom toolbar, the next *field* appears automatically.



6.1.3 Scoring a Single Slide

1. Open the **ImmunoScreener** *project*.
2. Right-click the slide that you want to score, select **Final Score** from the shortcut menu and select the score.



6.2 Marking Slides

You can flag slides in **ImmunoScreener** *projects*, for example to mark the ones that you have already scored.

1. Start the **ImmunoScreener** *project*.

2. Right-click the slide that you want to flag and select **Marked** from the shortcut menu.

7 Exporting Information from an ImmunoScreener Project

7.1 Exporting Data from an ImmunoScreener Project to a CSV File

In the export menu, you can export your added values and scores to a CSV (comma-separated value) file that you can open in a spreadsheet, or import into a database. You can export individual *grid field* values (data about this project) or you can export the data from this project into a long-term CSV file (a cumulative data file).

- Open the **ImmunoScreener** *project*.
- From the menu, select **Export** and select the data you want to export from the **ImmunoScreener** *project*:
  - **Full export**: data from the **ImmunoScreener** *project*, including a summary line, exported into its own CSV file.
  - **Cumulative export**: summary line appended to an existing CSV file where you compile longitudinal or otherwise cumulative data.

7.2 Exporting an HTML File

Tip!

ImmunoScreener does not export the scores to the HTML file. Add the scores to the *annotation* label before you export the *ImmunoScreener Gallery* and the score will be visible above each *annotation*.

1. Open the **ImmunoScreener** gallery.
2. Click on the top panel and select **Save to HTML** from the shortcut menu.

7.3 Exporting an image

Listed images can be exported at arbitrary magnification into a HTML file. You can also save all listed pictures as image files (BMP, JPEG or PNG).

1. Open the **ImmunoScreener** gallery.

2. Click on the panel and select **Save to Picture(s)** from the shortcut menu.

3. Set the magnification level and the format and then save the pictures.

7.4 Exporting One Annotation as a Picture from an ImmunoScreener Gallery

Listed *annotation*s can be exported at arbitrary magnification into a HTML file, or you can save all listed *annotation*s as image files (BMP, JPEG or PNG).

1. Open the **ImmunoScreener** gallery.

2. Right-click the *annotation* that you want to export.

3. Set the magnification level and the format and save the picture.

8 Working with ImmunoScreener in Multi-User Mode

If several users are assigned to an **ImmunoScreener** *project*, the application automatically creates a *Consolidator* user, who can see the scores entered by all other users and can finalize the score into one consolidated report.

Consolidator's Scoring offers the following options to *consolidator*s:

- The C*onsolidator* can see and score only those *field*s (*grid* cells) that received the same score from all users.
- The *Consolidator* can decide whether to take the lower or the upper median if there are even-numbered users assigned to the project.

9 Using HistoQuant and ImmunoScreener Together

You can use **ImmunoScreener** and **HistoQuant** together to perform measurements regarding the *marker*s used on the slides. If you first score a slide set with **ImmunoScreener**, you can create and run a **HistoQuant** algorithm on the *grid annotation*s to cross-check the manual scores or to support the *Consolidator*'s decisions in hard-to-decide cases. For example:

1. Evaluate the cuts from the specimen with possibly cancerous cells and score them manually as positive/negative in **ImmunoScreener**.

2. Create an algorithm on an *annotation* with easy-to-decide positive/negative *stain*s in **HistoQuant** for the same positive/negative parameter and run the algorithm on all *field*s in the **ImmunoScreener** *project*.

3. Compare the results from the manual scoring and from the **HistoQuant** algorithm and adjust the manual scores or refine the **HistoQuant** algorithm as necessary.

If you first run the **HistoQuant** algorithm on the *grid field*s or *annotation*s inside the *field*s, the **HistoQuant** measurements make it is easier for the *Evaluator*s to score the **ImmunoScreener** *Gallery*.